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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Continuing Prosecution Application Filed Herewith

Serial No.: 09/186,775

Filing Date: November 6, 1998

Applicant: DNA Plant Techology Corporation

Title: MATERIALS AND METHODS FOR HYBRID SEED PRODUCTION

Examiner: G. Helmer
Group Art Unit: 1638

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RESPONSE TO OFFICE ACTION

This Response to Office Action is being submitted concurrently with a Continuing Prosecution Application, and is in response to the Office Action dated December 3, 2002, the period for response to which has been extended to June 3, 2003 by the filing herewith of a Petition for a three-month extension of time and payment of the required fees.

Claims 1, 2, 4, 6, 7, 11, 12, 14-16, 18, 20, 21, 25, 26 and 28-37 stand rejected under 35 U.S.C. § 112, first paragraph. The Office Action contends that recombinase systems are unpredictable for generating particular phenotypes, referring also to the potential production of chimeric phenotypes, and thus Applicant has not provided sufficient guidance to practice the claimed methods utilizing recombinase systems. Applicant has carefully considered this position, and respectfully requests that the rejection be withdrawn in view of the following.

The presently claimed invention provides a two-component system to impair cellular function in plants. The system involves two polypeptides that are encoded by first and second

expression cassettes at the same locus on each of two homologous chromosomes. Each of the first and second expression cassettes is individually functional, and produces separate but complementary amino acid subsequences of a single functional nuclease, but neither subsequence alone provides the desired impairing effect. The desired impairing effect is obtained only by a combination of the complementary nuclease amino acid subsequences in a single cell. Nucleases have a known mechanism of action, and therefore the effect of nucleases in the plant cell is in fact highly predictable: nucleases degrade RNA and DNA that is essential for cell survival.

Applicant submits that one of ordinary skill in the relevant art, at the time of the filing of the above-captioned application, would have found the application specification to sufficiently enable that person to achieve the system of the invention. Moreover, that person of ordinary skill would understand, by combining the teachings known in the art at the time with the teachings in the Applicant's specification, how to achieve predictable and efficient impairing effects. Thus, despite the concerns raised in the Office Action, Applicant submits that the present specification quite adequately would have enabled one of skill in the art to predictably and efficiently utilize the claimed system.

In this regard, Applicant emphasizes that at the time of the filing of the above-captioned application, it was routine for one of ordinary skill in the art to transform a plant using site-specific recombinase systems to make single insertions which result in plants that incorporate only the desired transferred gene, and do not incorporate non-desired material. One of skill in the art, wanting to achieve a certain phenotype, could use the teachings of the art to delete a particular nucleotide or nucleotides using a site-specific recombinase system. At the time of filing, there were two main recombinase systems known to those of skill in the art: (1) the flp/frt system, and (2) the Cre/*lox* system. These systems are precise and conservative, and do not result in the loss of or alteration of nucleotides in the recombinant site, and thus may be used to create transgenic plants having particular nucleotides therein; by choosing the nucleotide to be transferred with a particular phenotype in mind, one of skill could relatively simply and predictably create a particular phenotype.

In support of Applicant's position, submitted herewith are copies of the following publications, which were available at the time the above-captioned application was filed, and which illustrate use of the Cre/*lox* system to create predictable phenotypes:

1. Dale, Emily C. and Ow, David W., "Gene transfer with subsequent removal of the selection gene from the host genome", *Proc. Natl. Acad. Sci. USA*, Vol. 88, pp. 10558-10562, December 1991; and

2. Qin, Minmin, Bayley, Chistopher, Stockton, Tamlyn and Ow, David W., "Cre recombinase-mediated site-specific recombination between plant chromosomes", *Proc. Natl. Acad. Sci. USA*, Vol. 91, pp. 1706-1710, March 1994.

Each of these references illustrates the use of the Cre/*lox* system for introducing a gene known to convey a certain phenotype. In the case of Reference No. 1, the firefly luciferase gene is introduced into the tobacco genome by using the *hpt* gene as a linked selectable marker. The *hpt* is flanked by recombination sites from the bacteriophage P1 Cre/*lox* recombination system, and the *hpt* is then excised from the plant genome by the Cre recombinase. Reference No. 2 teaches how to insert two different constructs into the tobacco genome, and states that progeny exhibit 67-100% co-transmission of the inserted transgenes, a percentage that indicates the highly predictable and reliable nature of recombinase systems in general that is known to those of ordinary skill in this art.

Additionally, please see U.S. Patent No. 5,658,772, issued August 19, 1997 and entitled "Site-specific recombination of DNA in plant cells", which illustrates that prior to filing of the above-captioned application, the use of site-specific recombination of DNA was routine, predictable and efficient. For example, in '772 see Examples 12 and 13 which show use of the Cre-*lox* recombination system for the disruption of seed development. See also Example 11, which illustrates the excision of a chimeric gene which causes male sterility, for the purpose of restoring fertility.

With respect to the concern stated in the Office Action about chimeric phenotypes, Applicant notes the following. Most if not all of the progeny produced by this method will contained the desired phenotype, and thus the process is considered highly efficient. Although the Examiner is correct in recognizing that initially not all cells of the plant may have the same genetic structure, because in some of the cells excision of the non-desired polynucleotide might not have been accomplished, practically speaking this is easily overcome. This is overcome by growing seeds from this plant, and then collecting seeds only from the progeny that exhibit the desired phenotype. All progeny thereafter would now have the desired phenotype.

The following describes how the method could be used:

1. A construct is created, consisting of an intervening second expression cassette surrounded by two *lox* recombinase sites, appropriately positioned between the promoter and coding sequences of the first expression cassette.

2. This system is then introduced into plants.

3. The plants are then screened for those that have a single copy of that second expression cassette, via a method known to one of ordinary skill at the time of filing, such as Southern hybridization, PCR, etc.

4. Seed are produced from lines with only a single copy, to get a plant line with suitable characteristics for subsequent treatment with Cre recombinase.

5. In another separate plant, the gene that constitutively expresses the Cre recombinase is introduced, to produce another line.

6. The plant lines produced in steps 4 and 5 above are then crossed, and seed is subsequently harvested from the cross progeny, knowing that the majority of the seed will have cleanly excised the intervening second expression cassette, leaving behind a functional first expression cassette with one remaining *lox* site.

7. The resulting plants of this next generation are screened using Southern hybridization, PCR, etc. for the absence of Cre (through segregation of the Cre locus) and the excision of the intervening cassette. Progeny with only the functional first expression cassette, and one remaining *lox* site, are thereby identified.

8. A plant of a line with the functional first expression cassette, from step 7, is crossed with a plant of a line having the construct of step 1, thereby generating lines having the structure set forth in claim 1 of the present application.

In conclusion, Applicant believes that the position in the Office Action is incorrect, and that the specification is sufficiently enabling. Thus, reconsideration and withdrawal of the rejection of the claims, and issuance of a Notice of Allowance, is most respectfully requested.

The Examiner is invited to telephone Applicant's undersigned representative if she believes that it would in any way facilitate prosecution of the application.

Dated: June 2, 2003

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I hereby certify that this paper and all documents and any fee referred to herein are being deposited on the date indicated above with the U.S. Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10, postage prepaid and addressed to Mail Stop CPA, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

6/2/03

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